Research Square

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Comparison of Allogenic and Autogenic Implantations of Dedifferentiated Fat Cells On Monoclonal Antibody 1-22-3-Induced Glomerulonephritis in Rats

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Abstract

Background

We established an adipogenic progenitor cell line derived from mature adipocytes and named these cells dedifferentiated fat (DFAT) cells, which have been shown to have characteristics very similar to those of mesenchymal stem cells (MSCs). The potential application of DFAT cells to support cell-based therapies for regenerative and immunosuppressive therapies has been suggested. The present study was designed to address beneficial ways that DFAT implantation can be used clinically as immunosuppressive therapy to treat immunological glomerulonephritis.

Methods

We evaluated distribution of DFAT cells after intravenous injection through the tail vein in Wistar rats. We examined effects of allogenic implantation of DFAT cells on BrdU incorporation into kidney from rats with monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis. We compared effects of allogenic and autogenic implantations of DFAT cells on excretion of urinary protein, renal function, and glomerular and nephrotubular injuries in these rats, and serum levels of tumor necrosis factor-stimulated gene-6 (TSG-6), and expression of TSG-6 mRNA in kidney.

Results

The allogenic implantations of DFAT cells trapped in lung improved excretion of urinary protein and renal function, and significantly suppressed glomerular and nephrotubular injuries in the rats with mAb1-22-3-induced glomerulonephritis compared with the autogenic implantations. The allogenic implantation of DFAT cells increased serum levels of TSG-6 especially in mAb 1-22-3-induced glomerulonephritis and significantly increased the expression of TSG-6 mRNA in kidney compared to the autogenic implantation.

Conclusion

These findings suggest that allogenic implantation of DFAT cells could be clinically useful immunosuppressive therapy for immunological glomerulonephritis.

Background

Despite the progress made in the development of medicines for chronic renal dysfunction, it cannot be cured with medicine, and thus, regenerative medicine has been expected as a novel therapy for this condition. As a source of cells for regenerative medicine, mesenchymal stem cells (MSCs) have been considered as a candidate to treat kidney disease. Recently, as an alternative source of MSCs, adipose tissue-derived cells such as adipose-derived stromal cells (ASCs) can be isolated, which are not a single

lineage of cells containing a variety of cell types [1]. We established an adipogenic progenitor cell line derived from mature adipocytes and named them dedifferentiated fat (DFAT) cells [2]. The cell surface antigen profile of DFAT cells has been shown to be very similar to that of bone marrow MSCs [3]. In contrast to ASCs, DFAT cells originate from a fraction of highly homogeneous mature adipocytes. Clonally expanded DFAT cells show the ability to differentiate into multiple mesenchymal cell lineages, indicating that DFAT cells represent a type of multipotent progenitor cell and suggesting that DFAT cells support their potential application to cell-based therapies. This property of DFAT cells will likely lead to higher safety and efficacy for clinical cell therapies.

It has been reported that implantations of the adipose-derived MSCs effectively improved ischemic reperfusion injury in kidney as acute kidney injury [4]. We also demonstrated that implantations of DFAT cells have been reported to be effective in improving renal injury [5]. In addition, we have demonstrated that implantations of DFAT cells have been effective in improving myocardial infarction [6], urethral sphincter dysfunction [7], osteoporosis [8], intervertebral disc regeneration, [9] and osteochondral defect [10, 11]. Thus DFAT cells are expected to be an effective cell source for regenerative medicine of mesenchymal organ dysfunctions.

Implantations of MSCs have been established to exert anti-inflammatory and immunosuppressive effects. Systematic infusion of MSCs has been reported to suppress graft rejection in animal models [12]. Further, the implantation of MSCs has been investigated in clinical studies in which MSCs were reported to effectively inhibit graft-versus-host disease [13].

We have also shown that DFAT cells have potential immunosuppressive effects. The systematic implantation of DFAT cells effectively ameliorated antibody-induced glomerulonephritis through immunosuppressive effects accompanied by the suppression of macrophage infiltration and increased the production of serum and renal tumor necrosis factor-stimulated gene-6 (TSG-6), which improved antibody-induced renal degeneration. These findings suggest that DFAT cells can potentially be a suitable cell source for the treatment of immunological progressive renal diseases [14]. In these implantations, we used allogenic DFAT cells for the immunosuppressive therapies.

For immunosuppression, MSCs can be implanted as autografts or allografts. Interestingly, allograft implantation of MSCs showed more beneficial effects than autograft implantation for the immunosuppression of graft-versus-host disease [15] and for chronic rejection in the solid organ transplant setting [16].

In the present study, to address beneficial ways that DFAT implantation can be used clinically as immunosuppressive therapy to treat immunological glomerulonephritis, we compared the effects of allograft and autograft implantations of DFAT cells on monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis in rats.

Methods

Antibodies

A hybridoma-producing mouse anti-rat Thy 1.1 mAb 1-22-3 (IgG3) was prepared by immunization of BALB/c mice with collagenase-treated fresh rat glomeruli. Ascitic fluid containing mAb 1-22-3 was produced in BALB/c mice primed with 2,6,10,14-tetramethylpentadecane (Sigma Chemical, St. Louis, MO) and injected intraperitoneally with the hybridoma. The obtained fluid was subjected to 50% ammonium sulfate (Kanto Chemical, Tokyo, Japan) precipitation, and the obtained immunoglobulin-rich fraction was dialyzed against phosphate-buffered saline (PBS, Kanto Chemical).

Animals and Ethics

Wistar rats were obtained from (Sankyo Laboratory, Tokyo, Japan).This investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, 1996). Euthanasia for rats was performed with carbon dioxide inhalation. The carbon dioxide flow rate displace 30% to 70% of the cage volume per minute.

Preparation of DFAT cells from adipose tissue

Around one g of epidydymal adipose tissue from six male Wistar rats was treated with collagenase and centrifuged. Adipocytes were isolated from the top layer. More than 99% of the isolated cells were mature lipid-filled adipocytes. The mature adipocytes floating on top of the culture medium attached to the upper surface of the culture flasks within a few days. Approximately 10–20% of the adherent cells flattened out by day 3 and changed to a spindle-shaped morphology by day 7. The cells subsequently entered a proliferative log-phase upon inversion of the flasks and changing of the media and reached confluence by day 14. During this stage, the cells lose their lipid droplets completely and exhibit the fibroblast-like morphology of DFAT cells. Six different DFAT cells from six Wistar rats, and combined them as allogenic DFAT cells.

Experimental protocols (Figure 1)

Experiment 1: Distribution of DFAT cells. DFAT cells from Wistar rats were labeled with a Qtracker® Cell Labeling Kit (Molecular probes, Life Technology, Tokyo, Japan). In total, 10^6 labeled DFAT cells were injected through the tail vein in Wistar rats. At 3 hours and one week after the injection, kidney, aorta, liver, and lungs were removed and fixed in 3% formalin (Kanto Chemical) in PBS and embedded in paraffin.

Experiment 2: Effects of implantation of DFAT cells on mAb1-22-3-induced nephritis. In all male Wistar rats weighing 250 g, the right kidney was nephrectomized. Rats were injected with 1.0 mL of saline containing 0.5 mg of mAb 1-22-3 through the tail vein at 7 days after nephrectomy. Thirty-five days after the nephrectomy, 1.0 mL of saline or 10^6 DFAT cells in 1.0 mL of saline were injected through the tail vein. Fifty-six days after the nephrectomy, 5-bromo-2-deoxyuridine (BrdU, Sigma Chemical) (0.1 mg/g body weight) was injected through the tail vein. Sixty-three days after the nephrectomy, all rats were killed and

the left kidney was removed. BrdU incorporation into kidney was determined at 28 days after implantation of allogenic DFAT cells.

Experiment 3: Effects of implantation of autogenic and allogenic DFAT cells on mAb 1-22-3-induced nephritis. In Wistar rats, the right kidney was nephrectomized. At 7 days after nephrectomy, rats were injected with 1.0 mL of saline containing 0.5 mg of mAb 1-22-3 through the tail vein. Thirty-five days after nephrectomy, 1.0 mL of saline or 10^6 autogenic DFAT cells prepared from identical Wistar rats or allogenic DFAT cells prepared from other identical Wistar rats in 1.0 mL of saline were injected through the tail vein. Sixty-three days after nephrectomy, all rats were housed in metabolic cages, and urinary protein excretion was determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA). Serum blood urea nitrogen (BUN) and creatinine were measured by SRL, Inc. (Wako, Saitama, Japan). Serum levels of TSG-6, and expression of TSG-6 mRNA in kidney were determined at 28 days after implantation of DFAT cells. All rats were then killed and the left kidney was removed.

Determination of BrdU incorporation into kidney

Removed kidneys were immediately frozen in liquid propane cooled to −196°C by liquid nitrogen. Frozen kidney slices were cut into 4-μm-thick cryostat sections. Sections were rinsed 3×10 min in 50 mM NH_4Cl (Kanto Chemical)/PBS to wash out the glutaraldehyde fixative and to reduce background staining. After pretreatment in 5% normal goat serum in PBS, the cryostat sections were incubated overnight in a humidified chamber at 4°C with mouse monoclonal anti-BrdU clone 3D4 (BD Biosciences Pharmingen, San Diego, CA, USA) 1:300, diluted in PBS-1% BSA. After incubation with primary antibodies, sections were rinsed three times with PBS and covered for 1 h at room temperature in the dark with the appropriate secondary antibodies coupled to fluorescein isothiocyanate (FITC, Sigma Chemical).

Morphological and immunohistological analysis

The 3-mm paraffin sections of removed renal cortex were stained with hematoxylin and eosin. Renal cortical thickness was measured under high magnification (×400). The glomerular injury score (GIS) was obtained by the following formula: $[(0 \times n0) + (1 \times n1) + (2 \times n2) + (3 \times n3) + (4 \times n4)]/50$. To semi-quantify the tubulointerstitial area, 20 areas of renal cortex were randomly selected. The percentage of each area that showed sclerofibrotic change was estimated and assigned a score of 0, normal; 1, involvement of <10% of the area; 2, involvement of 10–30% of the area; 3, involvement of >30–50% of the area; or 4, involvement of >50% of the area. The tubulointerstitial injury score (TIS) was similarly calculated as $[(0 \times n0) + (1 \times n1) + (2 \times n2) + (3 \times n3) + (4 \times n4)]/20$.

Determination of serum TSG-6

Levels of TSG-6 in blood serum were detected with a multi-detection microplate reader using a double-antibody sandwich ELISA kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocols. The concentrations of TSG-6 were normalized to the total protein content.

RNA extraction and real-time PCR

Total RNA was extracted from renal cortex and medulla with TRIzol reagent (Life Technologies) according to the manufacturer’s instructions. Total RNA (1 μg) was reverse transcribed into cDNA with random 9-mers with an RNA PCR Kit (AMV) Ver. 3.0 (Takara Bio, Ohtsu, Japan). Real-time quantitative PCR was performed with diluted cDNA using a FastStart TaqMan Probe Master (Roche Applied Science) and SYBR Select Master Mix (Life Technologies) in an ABI 7500 sequence detector (Life Technologies) according to the manufacturer’s instructions. All assay-on-demand primers and the TSG-6 probe were purchased from Life Technologies. Real-time PCR data were analyzed with standard curves and normalized to 18S ribosomal RNA with its specific primer sets (5' and 3' primers: 5'-CGGCTACCACATCCAAGGAA-3' and 5'-GCTGGAATTACCGCGGCT-3'). Correlation coefficients for the standard curves were all **>** 0.90.

Statistics

Values are reported as the mean ± SE. Two-way ANOVA with the Bonferroni/Dunn procedure as a post-test was also used. A value of $p < 0.05$ was considered to be statistically significant.

Results

Distribution of transplanted DFAT cells

In total, 10^6 Qtracker-labeled DFAT cells were implanted through the tail vein in Wistar rats. Seven days after injection, these DFAT cells were trapped mainly in the lung and were not delivered into the kidney, aorta, and liver (Figure 2).

Effects of implantation of DFAT cells on BrdU incorporation in mAb 1-22-3-induced nephritis

Figure 3 shows that incorporation of BrdU was enhanced in the nephrotubulus and glomerulus of kidney with mAb 1-22-3-induced nephritis. Implantation of allogenic DFAT cells markedly reduced the incorporation of BrdU in mAb 1-22-3-induced nephritis.

Effects of allogenic and autogenic implantations of DFAT cells on urinary protein and renal function in mAb1-22-3-induced nephritis

Implantations of allogenic and autogenic DFAT cells suppressed increased urinary protein excretion in the Wistar rats with mAb1-22-3-induced nephritis. The allogenic implantation inhibited the urinary protein excretion rather more than did the autogenic implantation but not significantly so (Figure 4a). Implantations of allogenic and autogenic DFAT cells significantly ($p < 0.05$) suppressed increased serum levels of BUN and creatinine in the Wistar rats with mAb1-22-3-induced nephritis. The allogenic implantation inhibited the serum levels of BUN and creatinine rather more than did the autogenic implantation but not significantly so (Figure 4b).

Effects of allogenic and autogenic implantations of DFAT cells on renal injury in mAb1-22-3-induced nephritis

Injection of mAb1-22-3 significantly ($p < 0.01$) increased the GIS and TIS of kidneys from Wistar rats. Implantations of allogenic and autogenic DFAT cells significantly suppressed increases in GIS and TIS in the Wistar rats with mAb1-22-3-induced nephritis. Suppression of the GIS and TIS by allogenic implantation was significantly ($p < 0.05$) greater than that by autogenic implantation (Figure 5).

Effects of allogenic and autogenic implantations of DFAT cells on TSG-6 in serum and kidney

Serum levels of TSG-6 were significantly ($p < 0.01$) higher with implantation of allogenic and autogenic DFAT cells in the Wistar rats with mAb1-22-3-induced nephritis than those in normal rats. There was no differences of serum levels of TSG-6 after implantation of allogenic and autogenic DFAT cells in normal rats and rats with mAb1-22-3-induced nephritis (Figure 6a). There was no difference of expression of TSG-6 mRNA between implantations of allogenic and autogenic DFAT cells in normal Wistar rats. Expression of TSG-6 mRNA were significantly ($p < 0.05$) higher with implantation of allogenic DFAT cells than with autogenic DFAT cells in Wistar rats with mAb1-22-3-induced nephritis (Figure 6b).

Discussion

We previously showed that the injection of mAb 1-22-3 induces chronic glomerulonephritis with infiltration of monocytes/macrophages and T cells and increases in the expression of interleukin (IL)-6, IL-10, and IL-12β mRNAs in glomeruli of kidneys in rats. The implantation DFAT cells decreased infiltrations of the monocytes/macrophages, whereas it increased the helper T cells and suppressed the elevated expressions of IL-6, IL-10, and IL-12β mRNAs. These findings indicate that the implantation of DFAT cells ameliorated the mAb 1-22-3-induced glomerulonephritis by immunosuppressive effects [14].

In the present experiments, we compared the effectiveness of the allogenic and autogenic implantations of DFAT cells on mAb 1-22-3-induced glomerulonephritis in rats. The allogenic implantation inhibited the urinary protein excretion and improved renal function in the rats. Its effectiveness was greater than that of the autogenic implantation of DFAT cells. Moreover, allogenic implantation strongly suppressed morphological glomerular and intestinal renal injuries in these rats compared with the autogenic implantation of DFAT cells.

These findings suggest that the immunosuppressive effects of the allogenic implantation of DFAT cells on immunological glomerulonephritis are stronger than those with the autogenic implantation. Concerning the effectiveness of allogenic implantations of MSCs on immunological kidney diseases, Reinders et al. [16] investigated the safety of allogenic implantation of MSCs in renal transplant recipients by comparing fibrosis in renal biopsies in terms of de novo human leukocyte antigen (HLA) antibody development, extensive immune monitoring, and renal function. They suggested that the allogeneic implantation of MSCs was safe in renal transplant recipients and effectively improved immunological rejections. Ma et al. [17] showed that the implantation of MSCs prolongs the survival of

mice with lupus nephritis. The implantation of MSCs significantly decreased B-cell activating factor accompanied by decreases in the production of anti-dsDNA autoantibodies and proteinuria. Thus, the implantation of allogenic DFAT cells can potentially be useful therapy for clinical immunological glomerulonephritis such as lupus nephritis.

In the present experiments, even though almost all DFAT cells injected through the tail vein were trapped mainly in the lung and did not reach the kidney, serum levels of TSG-6 were increased in the rats with mAb 1-22-3-induced glomerulonephritis, and the expression of TSG-6 mRNA was increased in the renal cortex and medulla of these glomerulonephritis rats. These findings indicate the systematic implantation of DFAT cells increased serum levels of TSG-6 especially in rats with glomerulonephritis and also increased the expression of TSG-6 mRNA in kidney from rats with the glomerulonephritis, even though no cells were delivered to the kidney. It has also been shown that intravenously infused MSCs were mostly trapped in lung and that they secrete an anti-inflammatory protein, TSG-6, which decreases inflammatory responses and improves injured organs [18]. TSG-6, a 30-kDa protein, is regulated by a number of signaling molecules such as TNF-α and IL-1 and the mechanical stimuli in mesenchymal cells [19]. Wang et al. [20] recently showed that MSCs trapped in the lung produce TSG-6 after intravenous injection, which induces anti-inflammatory and immunosuppressive effects. They also found that intravenous injection of conditioned medium of cultured MSCs increased serum levels of TSG-6 as well as tissue levels of TSG-6 in injured peritoneum [20]. In the present experiments, the systematic implantation of DFAT cells increased the serum levels of TSG-6 and the in the mAb 1-22-3-injected rats rather than those in normal rats. These findings suggest that the implanted DFAT cells trapped in lung abundantly produced TSG-6 in serum that also increased the generation of TSG-6 that ameliorated the mAb 1-22-3-induced glomerulonephritis rather than normal rats. Interestingly, expression of TSG-6 mRNA was significantly higher with implantation of allogenic DFAT cells than with autogenic DFAT cells in Wistar rats with mAb1-22-3-induced nephritis, even there was no difference of expression of TSG-6 mRNA with implantations of allogenic and autogenic DFAT cells in kidneys from normal rats. These findings suggest that the allogenic implantation of DFAT cells may enhance the expression of TSG-6 in the injured kidney with the mAb1-22-3.

It has been reported that in terms of the mechanisms underlying the induction of immunosuppressive and anti-inflammatory effects by lung-trapped MSCs and DFAT cells, these effects of the implantation of MSCs are associated with the secretion of soluble factors with paracrine actions that are mediated by exosomes. Exosomes are predominantly released from the endosomal compartment and contain miRNA, cytokines, and proteins from MSCs. Recent studies in animal models suggest that exosomes have significant potential as a novel alternative to whole-cell therapies [21]. Bruno et al. [22] showed that exosomes derived from MSCs improve acute tubular injury. Chaubey et al. [23 reported that implantation of MSCs improved experimental bronchopulmonary dysplasia in part via exosome-associated factor TSG-6. Thus, mechanisms of improvement of mAb 1-22-3-induced glomerulonephritis may be associated with the action of implanted DFAT cells to increase TSG-6 through exosomes, which may be enhanced in the immune-induced glomerulonephritis.

Conclusion

The systematic implantations of allogenic DFAT cells trapped in lung improved excretion of urinary protein and renal function, and significantly suppressed glomerular and nephrotubular injuries in the rats with mAb1-22-3-induced glomerulonephritis compared with the autogenic implantations. The allogenic implantation of DFAT cells increased serum levels of TSG-6 especially in mAb 1-22-3-induced glomerulonephritis and significantly increased the expression of TSG-6 mRNA in kidney compared to the autogenic implantation. These findings suggest that allogenic implantations of DFAT cells can potentially be clinically useful as immunosuppressive therapy for various types of immunological glomerulonephritis such as lupus nephritis and ANCA-associated glomerulonephritis.

Abbreviations

ASCs
adipose-derived stromal cells
BrdU
5-bromo-2-deoxyuridine, BUN:blood urea nitrogen
DFAT
dedifferentiated fat
GIS
glomerular injury score
HLA
human leukocyte antigen
IL
interleukin
mAb
monoclonal antibody
MSCs
mesenchymal stem cells
PBS
phosphate-buffered saline
TIS
tubulointerstitial injury score
TSG-6
tumor necrosis factor-stimulated gene-6.

Declarations

Ethics approval and consent to participate

This investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, 1996). This study was approved by the ethics committee of Nihon University School of Medicine (No. AP10M054).

Consent for publication

Not applicable.

Conflict of interest

The all authors have no conflicts of interest to declare.

Availability of data and materials

Data are available from the authors upon reasonable request (fukuda.noboru@nihon-u.ac.jp).

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Authors' contribution

Every one of the authors actively participated in planning the study and interpreting the study results. NF contributed design of this study. TKM, KU and SS performed practical animal experiments. TRM and KK contributed preparing DFAT cells. TKM contributed interpretation of data. ME conducted the morphological evaluation of kidney. TKM, NF and MA interpreted the data and revised the manuscript. All authors have approved the final version of the manuscript and agree with its submission to *BMC Nephrology.*

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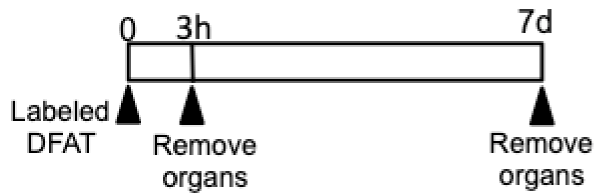
Figures

Figure 1

Experiment 1



Experiment 2

Experiment 3

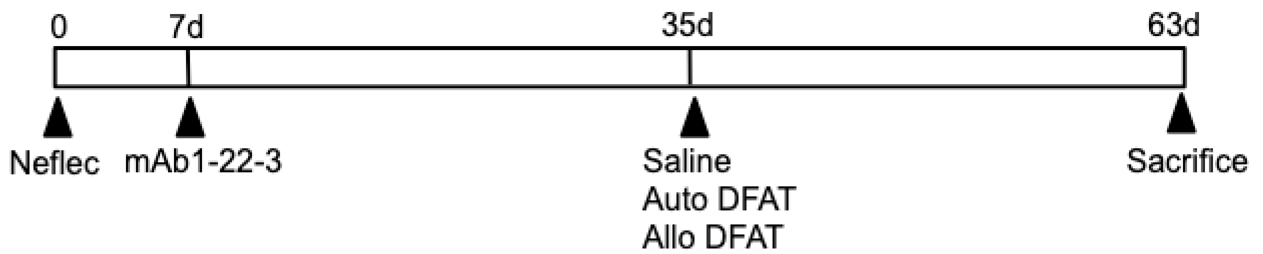


Figure 1

Schematic of the experimental protocols in this study

Figure 2

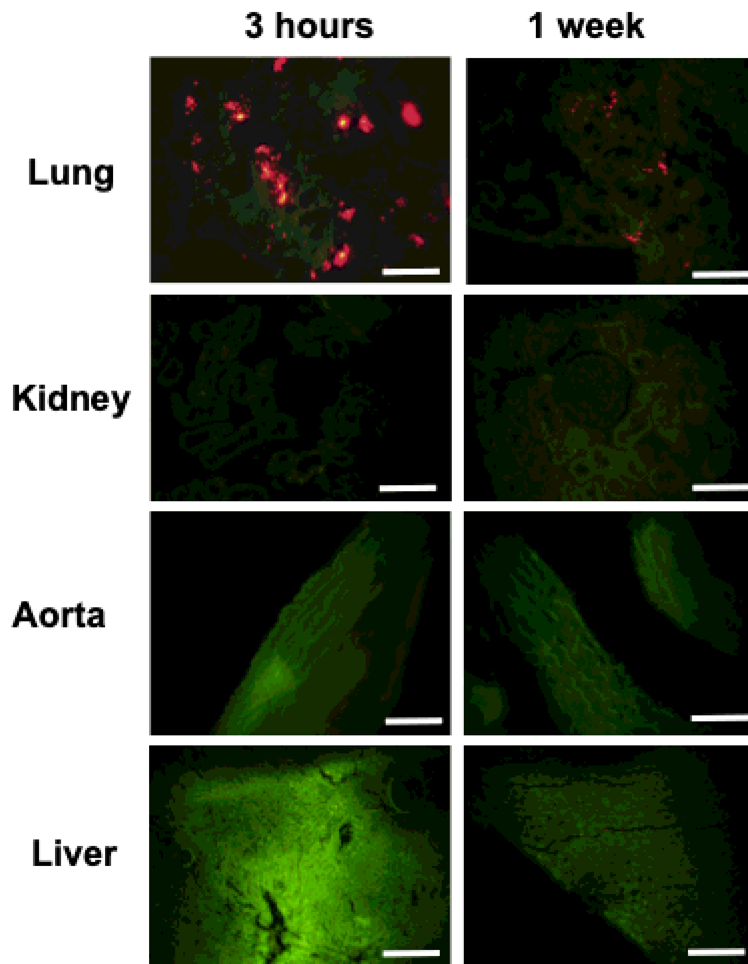


Figure 2

Distribution of dedifferentiated fat (DFAT) cells by intravenous injection. In total, 106 labeled DFAT cells were injected through the tail vein in Wistar rats. Three hours and one week after the injection, kidney, aorta, liver, and lungs were removed and fixed in 3% formalin in PBS and embedded in paraffin. Images were obtained with a digital imaging system. Bar = 50 μm

Figure 3

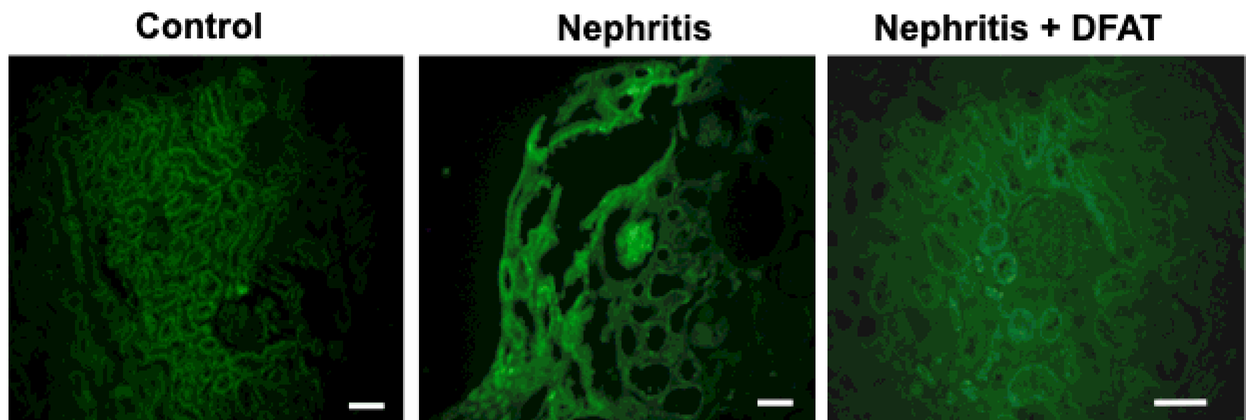


Figure 3

Effects of implantation of dedifferentiated fat (DFAT) cells on 5-bromo-2-deoxyuridine (BrdU) incorporation into kidney from monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis rats. Wistar rats were injected with 0.5 mg of mAb 1-22-3 through the tail vein at 7 days after right nephrectomy. Thirty-five days after nephrectomy, saline or 106 DFAT cells were injected through the tail vein. Fifty-six days after nephrectomy, BrdU (0.1 mg/g body weight) was injected through the tail vein. Sixty-three days after nephrectomy, all rats were killed and the left kidney was removed. BrdU incorporation into kidney was determined by immunohistochemistry with BrdU antibody and secondary FITC labeling. Bar = 50 μm

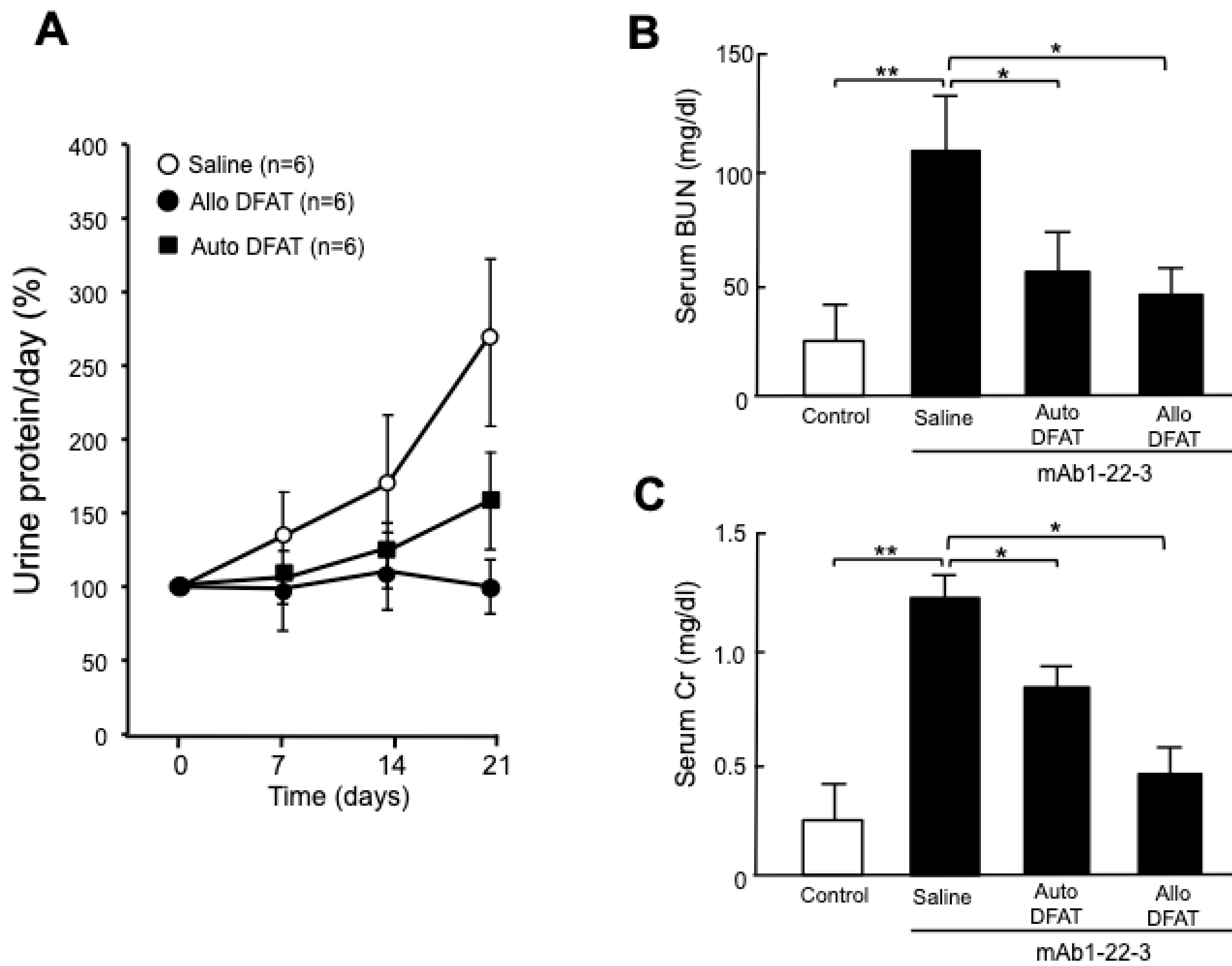


Figure 4

Effects of allogenic and autogenic implantations of dedifferentiated fat (DFAT) cells on proteinuria and renal function in monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis rats. Thirty-five days after right nephrectomy, saline or 106 autogenic (Auto) or allogenic (Allo) DFAT cells were injected into normal Wistar rats (Control) and mAb 1-22-3-induced glomerulonephritis rats through the tail vein. Sixty-three days after nephrectomy, the left kidney was removed. Urinary protein excretion (A) and serum levels of BUN (B) and creatinine (Cre) (C) were measured. Data are the mean ± SEM (n=6). *p<0.05 and ** <0.01 in the indicated columns

Figure 5

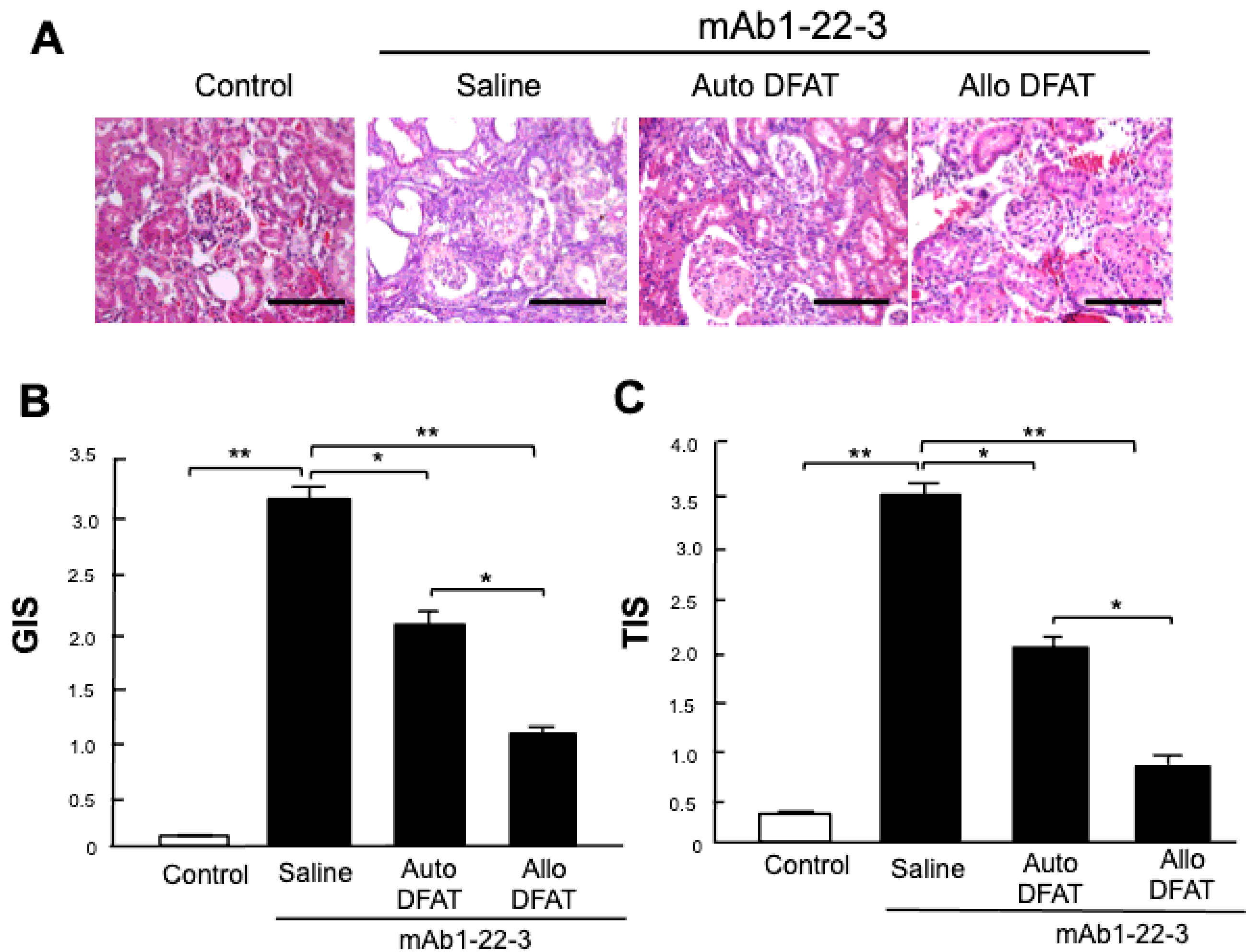


Figure 5

Effects of allogenic and autogenic implantations of dedifferentiated fat (DFAT) cells on degeneration of renal cortex in monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis. Thirty-five days after right nephrectomy, saline or 106 autogenic (Auto) or allogenic (Allo) DFAT cells were injected into normal Wistar rats (Control) and mAb 1-22-3-induced glomerulonephritis rats through the tail vein. Sixty-three days after nephrectomy, the left kidney was removed. A. The paraffin sections of removed renal cortex were stained with hematoxylin and eosin. Renal cortical thickness was measured under high magnification (×400). B. Glomerular injury score (GIS). C. Tubulointerstitial injury score (TIS). Data are the mean ± SEM (n=6). *p<0.05 and **p<0.01 in the indicated columns. Bar = 50 μm

Figure 6

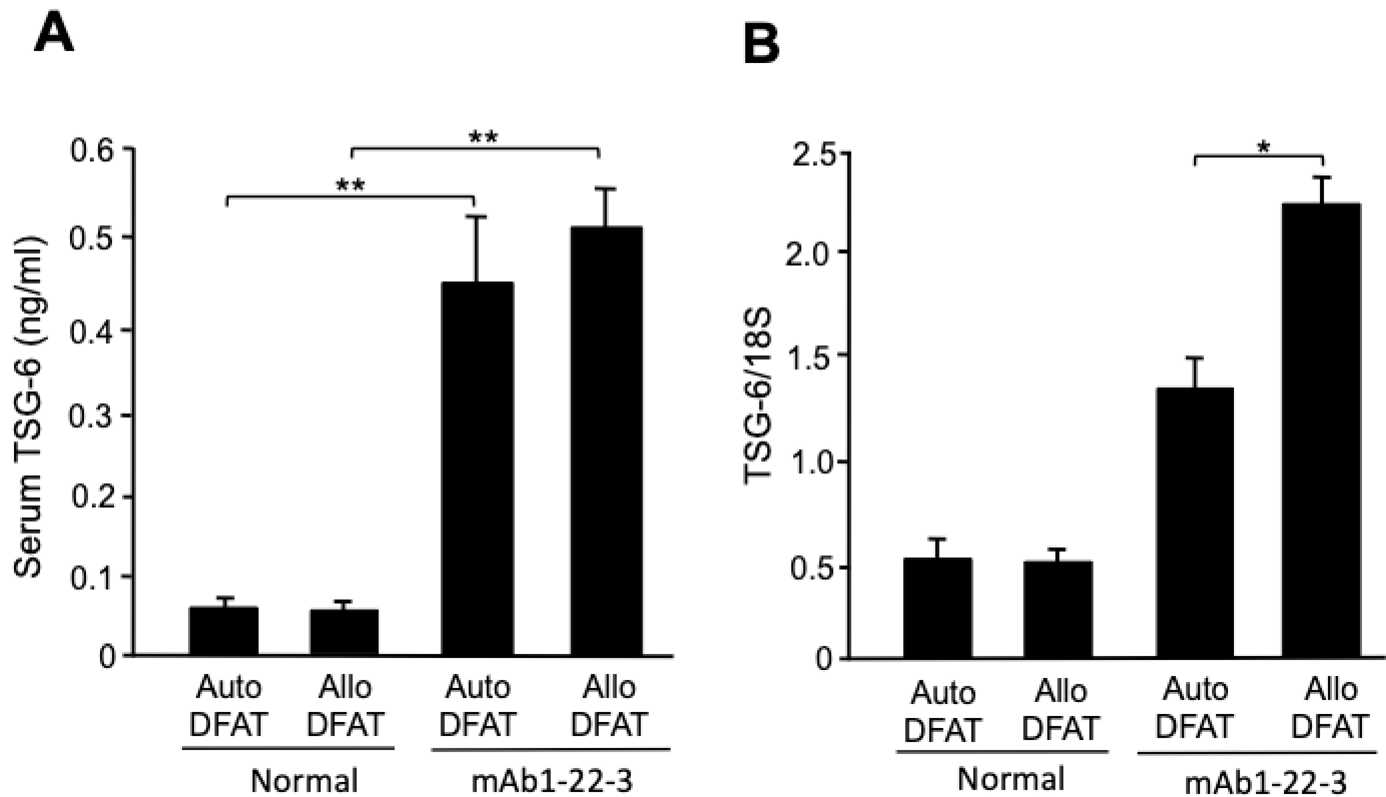


Figure 6

Effects of allogenic and autogenic implantations of dedifferentiated fat (DFAT) cells on (A) serum levels of tumor necrosis factor-stimulated gene-6 (TSG-6) and (B) expression of TSG-6 mRNA in kidney from monoclonal antibody (mAb) 1-22-3-induced glomerulonephritis rats. Number of 106 DFAT cells were injected into normal Wistar rats through the tail vein, and 106 DFAT cells were injected into mAb 1-22-3-induced glomerulonephritis rats through the tail vein. Serum levels of TSG-6 were determined by ELISA, and expression of TSG-6 mRNA in renal cortex was determined by real time-PCR analysis at 18 days after implantation of allogenic DFAT cells. Data are the mean ± SEM (n = 6). *p<0.05, **p<0.01 between indicated columns.